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VERIFICATION OF A TRANSLATION

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My name and post office address are as stated below;

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I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: July 31, 2006

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Use of a double-stranded ribonucleic acid to selectively inhibit expression of a given target gene

The invention concerns the use of a double-stranded ribonucleic acid (dsRNA) to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene. Furthermore, it concerns the use of such a ribonucleic acid to produce a medicament, a medicament and a method, to selectively inhibit expression of the aforementioned target gene in a cell.

A method to inhibit expression of a target gene in a cell is known from DE 101 00 586 C1, in which an oligoribonucleotide having a double-stranded structure is introduced into the cell. One strand of the double-stranded structure is complementary to the target gene.

It is known from Elbashir, S.M. et al., Nature 411 (2001), pages 494-498, that a short dsRNA in which three nucleotides are not complementary to the target gene hardly inhibits expression of a target gene. On the other hand, a completely complementary dsRNA induces effective inhibition of expression of the target gene.

From Holen, T. et al., Nucleic Acids Research 30 (2002), pages 1757-1766, it is known that inhibition of expression of a gene by short dsRNA by means of RNA interference is also possible with dsRNAs, one of whose strands exhibits one or two nucleotides that are not complementary to the target gene.

Many diseases and defects of cells result from an alteration of a gene, often a proto-oncogene, that is important to cells by one or few point mutations. The problem in treating such a

disease or such cells with the methods known to date is that inhibition of expression of the mutated gene often leads also to an inhibition of the unmutated gene. This is often associated with grave side effects.

The task of the present invention is to remove these shortcomings in accordance with the state-of-the-art. In particular, a use of a dsRNA to selectively inhibit expression of a target gene in a cell having a point mutation not found in an original gene is to be made available, in which expression of the original gene remains largely unaffected. Furthermore, a medicament and a method to selectively inhibit expression of a given target gene, as well as a use for the production of the medicament are to be made available. This task is solved by the features of Claims 1, 2, 17, and 31. Advantageous embodiments result from the features of Claims 3 to 16, 18 to 30, and 32 to 41.

According to the invention, a use of a double-stranded ribonucleic acid (dsRNA) to selectively inhibit expression of a given target gene in a cell having a point mutation not found in an original gene is intended, whereby an S1 strand of the dsRNA exhibits a region that is complementary to the target gene in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene. The invention further concerns the use of such dsRNA to produce a medicament to selectively inhibit expression of a given target gene in a cell having a point mutation not found in an original gene. In this invention, a nucleotide is "complementary" to the target gene or original gene when it can form a specific Watson-Crick base pair with a nucleotide that corresponds to it in its own sequence position

therein. The target gene is generally understood to be the DNA strand of the double-stranded DNA present in the cell that is complementary to a DNA-strand that serves as a matrix for transcription, including all transcribed regions. As a rule this is the sense strand. The S1 strand can thus be complementary to an RNA transcript formed during expression or its processing product, e.g., an mRNA. It can, for example be sufficient for the S1 strand to be complementary to a part of the 3'-untranslated region of the mRNA. However, the target gene can also be a part of a viral genome. The viral genome can also be the genome of a (+) strand RNA virus, in particular a hepatitis C virus.

The original gene can be any gene that deviates by only one or a few point mutations from the target gene that is to be inhibited. In general it is a wild-type gene. DsRNA is present when the ribonucleic acid that consists of one or two nucleic acid strands exhibits a double-stranded structure. Not all nucleotides of the dsRNA must exhibit canonical Watson-Crick base pairs within the dsRNA. The maximum possible number of base pairs is the number of nucleotides in the shortest strand contained in the dsRNA. The region that is complementary to the target gene can exhibit—in order of ascending preference—fewer than 25, 19 to 24, 21 to 23, and particularly 21 nucleotides. The S1 strand can exhibit—in order of ascending preference—fewer than 30, fewer than 25, and particularly 21 to 24 nucleotides. It has been shown that short dsRNAs are particularly effective in inhibiting expression of a target gene. Such dsRNAs are also called siRNAs (short interfering RNAs).

In selective inhibition, expression of the original gene is inhibited less than that of the target gene. Ideally,

expression of the original gene should remain largely unaffected. To this end, dsRNA that is not optimal to inhibit expression of the target gene is selectively used. Thus, a dsRNA can be prepared that has so little complementarity to the original gene that its expression remains largely unaffected. Adverse side effects resulting from inhibition of the original gene can be avoided or lessened.

Inhibition of expression by means of dsRNA preferably occurs according to the principle of RNA interference. The nucleotide that is not complementary to the target gene is preferably not located at the 3'-end or 5'-end of the region. Ideally, the non-complementary nucleotide is located in the middle portion of the region. The target gene may exhibit one or two point mutations not found in the original gene. In that case, use according to the invention to selectively inhibit expression of the target gene is particularly suitable to selectively inhibit only this expression, and not that of the original gene.

In one embodiment of the invention, the original gene is a proto-oncogene and the target gene is an oncogene derived from it. An oncogene is frequently differentiated only by a single point mutation from the cellular proto-oncogene that corresponds to it. For this reason, however, inhibition of the expression of the oncogene using conventional dsRNA usually inhibits expression of the corresponding proto-oncogene as well. This is often associated with such grave side effects that a use of conventional dsRNA to inhibit the target gene in an organism is virtually impossible.

The cell can be tumor cell. In one embodiment of the invention, one nucleotide of the region is not complementary

to the target gene, and two nucleotides of the region are not complementary to the original gene. Even such a small difference in the number of complementary nucleotides can be sufficient to almost completely inhibit expression of the target gene, while leaving expression of the original gene largely unaffected.

In one embodiment of the method, at least one base pair within the dsRNA is not complementary, i.e., the nucleotides of the base pair are not specifically paired according to Watson-Crick. By varying the number of non-complementary base pairs within the dsRNA, the effectiveness of the dsRNA can be modulated. Reduced complementarity within the dsRNA lessens its stability in the cell.

DsRNA preferably exhibits a single-stranded overhang consisting of 1 to 4, particularly 2 or 3 nucleotides at least at one end of the dsRNA. One end is a dsRNA region in which a 5'- and a 3'-strand end are present. DsRNA consisting only of the S1 strand accordingly exhibits a loop structure and only one end. DsRNA consisting of the S1 strand and an S2 strand exhibits two ends. Here, one end is formed in each case by a strand end on the S1 strand and one on the S2 strand. Single stranded overhangs reduce the stability of dsRNA in blood, serum, and cells, while simultaneously increasing the expression-inhibiting action of dsRNA. It is particularly advantageous for dsRNA to exhibit the overhang exclusively at one end, particularly at its end exhibiting the 3'-end of the S1 strand. The other end is then blunt in dsRNA that exhibits two ends, i.e., it lacks overhangs. Surprisingly, it has been shown that to increase the expression-inhibiting action of dsRNA, one overhang at one end of the dsRNA is sufficient and does not decrease stability to such an extent as occurs with

two overhangs. DsRNA with only one overhang has shown itself to be sufficiently stable and particularly effective in various cell culture mediums, as well as in blood, serum, and cells. Inhibition of expression is particularly effective when the overhang is located at the 3'-end of the S1 strand.

The S1 strand or an S2 strand possibly contained in the dsRNA can be complementary to the primary or processed RNA transcript of the target gene. The dsRNA may be present in a preparation suitable for inhalation, oral ingestion, infusion and injection, in particular for intravenous, intraperitoneal or intratumoral infusion or injection. The preparation can consist of the dsRNA and a physiologically tolerated buffer, particularly a phosphate buffered saline solution.

Surprisingly, it has been shown that dsRNA that has simply been dissolved and administered in such a buffer is taken up by the cell and inhibits expression of the target gene, without the dsRNA having had to be packaged in a special vehicle.

Preferably, dsRNA is present in a physiologically tolerated buffer, in particular a phosphate buffered saline solution, or surrounded by a micellar structure, preferably a liposome, a virus capsid, or a capsoid. The dsRNA can be administered orally, by means of inhalation, infusion, or injection, in particular by intravenous, intraperitoneal, or intratumoral infusion or injection. Preferably, the dsRNA is administered to a mammal, preferably a human being, at a maximum dosage of 5 mg, particularly 2.5 mg, preferably 200 μg, most preferably 100 μg per kilogram body weight per day.

Furthermore, the invention concerns a medicament to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene, whereby the medicament contains a double-stranded ribonucleic acid (dsRNA) whereby an S1 strand of the dsRNA exhibits a region that is complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene.

Furthermore, according to the invention a method to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene is intended, whereby a double-stranded ribonucleic acid (dsRNA) is introduced into the cell, and an S1 strand of the dsRNA exhibits a region that is complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene.

With regard to other advantageous embodiments of the medicament and method that are the subject of this invention, see the above remarks.

In the following examples, the invention will be discussed on the basis of the figures. They show:

Figure 1 a graphic depiction of the inhibition of the expression of an HCV luciferase fusion protein by dsRNAs, which to a varying degree are complementary to a sequence of a target gene and

Figure 2 a graphic depiction of the inhibition of the expression of an HCV luciferase fusion protein by dsRNAs, which to a varying degree are complementary to a sequence of a target gene, and are partially formed from RNA strands that are not completely complementary to each other.

In order to make a reporter system, a 26-nucleotide-long sequence of a cDNA sequence that serves as a target gene and corresponds to the 3'-untranslated region of an HCV-RNA was fused with the open reading frame of the luciferase gene from the pGL3 expression vector. The pGL3 expression vector came from Promega Co., and is registered under Gene Accession No. U47296 with the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Bethesda, MD 20894, USA. Nucleotides 280 to 1932 were used as the luciferase gene. The 26-nucleotide-long sequence is a sequence that is present in many HCV genomes and their subtypes and that is highly preserved. The 26 nucleotides correspond to Nucleotides 9531 to 9556 of the HCV genome that is registered with the NCBI under Gene Accession No. D89815. They exhibit the following sequence:

5'-gtcacggct agctgtgaaa ggtccgt-3' (SEQ ID NO: 1).

The resulting fusion gene has been cloned as a BamHI/NotI DNA fragment in the eukaryotic expression plasmid pcDNA 3.1 (+) by Invitrogen GmbH, Karlsruhe Technology Park, Emmy Noether Str. 10, 76131 Karlsruhe, Catalogue No. V790-20. The resulting plasmid is designated p8.

The pCMVβGal plasmid from Clontech, Gene Accession No. U13186, NCBI, was used to control for transfection effectiveness. This

plasmid codes for the enzyme β-galactosidase and induces its expression.

The plasmid that contains the fusion gene, the plasmid that serves as the control, and various dsRNAs were introduced together by transfection into cells of the liver cell line HuH-7 (JCRB0403, Japanese Collection of Research Bioresources Cell Bank, National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158, Japan). Inhibition of expression of the luciferase gene that was induced by the dsRNAs has been determined in relation to the expression of the β-galactosidase gene.

The dsRNAs that were used exhibit the following sequences designated as SEQ ID NO:2 to SEQ ID NO:13 in the sequence listing:

HCV1+2, whose S1 strand is completely complementary to the HCV sequence in the fused HCV-luciferase gene:

S2: 5'- ACG GCU AGC UGU GAA AGG UCC GU-3' (SEQ ID NO:2)
S1: 3'-AG UGC CGA UCG ACA CUU UCC AGG -5' (SEQ ID NO:3)

HCV3+4, which is complementary neither to the HCV- nor to the luciferase sequence in the fused HCV-luciferase gene, and which serves as negative control:

S2: 5'- AGA CAG UCG ACU UCA GCC U GG-3' (SEQ ID NO:12)
S1: 3'-GG UCU GUC AGC UGA AGU CGG A -5' (SEQ ID NO:13)

HCV5+6, whose S1 strand is complementary to the HCV sequence in the fused HCV-luciferase gene, except for the nucleotide that is in bold:

S2: 5'- ACG GCU AGC UGU GAA **U**GG UCC GU-3' (SEQ ID NO:6)
S1: 3'-AG UGC CGA UCG ACA CUU **A**CC AGG -5' (SEQ ID NO:7)

HCV7+8, whose S1 strand is complementary to the HCV sequence in the fused HCV-luciferase gene, except for the two nucleotides that are in bold:

S2: 5'- ACG GC**A** AGC UGU GAA **U**GG UCC GU-3' (SEQ ID NO:8)
S1: 3'-AG UGC CG**U** UCG ACA CUU **A**CC AGG -5' (SEQ ID NO:9)

Luc1+2, whose S1 strand is completely complementary to a luciferase sequence in the fused HCV-luciferase gene, and which serves as positive control:

S2: 5'- CGU UAU UUA UCG GAG UUG CAG UU-3' (SEQ ID NO: 10)
S1: 3'-GC GCA AUA AAU AGC CUC AAC GUC -5' (SEQ ID NO: 11)

K3s+K3as, which is complementary neither to the HCV- nor to the luciferase sequence in the fused HCV-luciferase gene, and which serves as negative control:

S2: 5´- G AUG AGG AUC GUU UCG CAU GA-3´ (SEQ ID NO: 4)
S1: 3´-UCC UAC UCC UAG CAA AGC GUA -5´ (SEQ ID NO: 5)

HCV5+2, whose S1 strand is completely complementary to the HCV sequence, and whose S2 strand is complementary to the HCV sequence in the fused HCV-luciferase gene, except for the nucleotide that is in bold:

S2: 5'- ACG GCU AGC UGU GAA **U**GG UCC GU-3' (SEQ ID NO:6)
S1: 3'-AG UGC CGA UCG ACA CUU UCC AGG -5' (SEQ ID NO:3)

HCV1+6, whose S2 strand is completely complementary to the HCV sequence, and whose S1 strand is complementary to the HCV sequence in the fused HCV-luciferase gene, except for the nucleotide that is in bold:

S2: 5'- ACG GCU AGC UGU GAA AGG UCC GU-3' (SEQ ID NO:2)
S1: 3'-AG UGC CGA UCG ACA CUU **A**CC AGG -5' (SEQ ID NO:7)

HuH-7 cells were cultured in DMEM with 10% FCS. In preparation for transfection, 2 x 10^4 cells per well of a 96-well cell culture plate were seeded. The cells were transfected 24 hours after seeding by means of 110 µl transfection medium per well of the 96-well cell culture plate each, and cultured further in this total volume. Each transfection was done three times.

For that at first 3 µg of the pCMVβGal plasmid and 1 µg of the p8 plasmid were mixed. The transfection medium contained 0.25 µg of the plasmid mixture, and 200, 100, 50, 25, 12.5, or 0 nmol/l of one of the aforementioned dsRNAs per well.

"Gene Porter 2" from PEQLAB Biotechnologie GmbH, Carl Thiersch Str. 2b, D-91052 Erlangen, Catalogue No. 13-T202007 was used for the transfection in accordance with manufacturer instructions.

Next, the cells were incubated at 37°C and 5% CO_2. One day after transfection, 35 µl of fresh medium was added per well, and the cells were incubated for another 24 hours.

The effect of the dsRNAs that were used was determined by quantifying expressed β-galactosidase by means of "Galacto-Star" from Tropix Corp., 47 Wiggins Avenue, Bedford, MA 01730, USA, Catalogue No. BM100S, and the effect of the expressed

luciferase was determined by chemoluminescence reaction by means of "Luciferase" from Tropix Corp., Catalogue No. BC100L. For that cell lysates were made in accordance with manufacturer instructions, and from that 2 µl each was used per analysis to test for β-galactosidase and 5 µl each was used per analysis to test for luciferase. Chemoluminescence measurement was done in a Sirius Luminometer (Berthold Detection Systems GmbH, Bleichstr. 56-68, D-75173 Pforzheim, Germany). The relative activity of luciferase as a measurement of expression is determined in each case by dividing the luciferase value of chemoluminescence by the β-galactosidase value. An average is calculated for every 3 values determined in this way. The average for cells transfected without dsRNA is arbitrarily defined as 1.0. The other averages are expressed as a ratio with that value, and these are depicted graphically in Figures 1 and 2.

Luc1+2 (positive control) led to the most marked inhibition of luciferase activity (Figures 1 and 2). In the presence of HCV1+2, which was completely complementary to the target sequence for the reporter plasmid, a clear reduction in luciferase activity is also discernible (Figures 1 and 2). Luciferase activity increased with decreasing concentration of HCV1+2. The HCV5+6 oligonucleotide, which is not complementary to the target sequence by one nucleotide, is approximately as effective in inhibiting luciferase as HCV1+2, particularly at low concentrations (Figure 1). As far as the specificity of this dsRNA is concerned, this means that it is not enough for the dsRNA to be complementary to the target gene, while it is not complementary to the original gene by one nucleotide, to specifically inhibit expression of the target gene when compared to expression of the original gene.

HCV7+8 inhibits expression of luciferase both at high and at low concentrations only to the same degree as the negative controls HCV3+4 and K3S+K3AS (Figures 1 and 2). The scant inhibition of luciferase activity is to be seen as a nonspecific effect. As far as the specificity of this dsRNA is concerned, this means that it is enough for the dsRNA to be complementary to the target gene, or off by only one nucleotide, but not be complementary to the original gene by two nucleotides, to specifically inhibit expression of the target gene when compared to expression of the original gene.

In HCV5+2, one nucleotide in the S2 sense strand is not complementary to the S1 antisense strand, whereby the S1 antisense strand is completely complementary to the target gene. This dsRNA is as effective as LUC1+2 and HCV1+2 (figures 1 and 2). This is surprising because complementarity within dsRNA that is reduced by one base pair would lead one to expect a lesser stability of the dsRNA in the cell and therefore a lesser effectiveness.

In HCV6+1, one nucleotide in the S1 antisense strand is not complementary to the S2 sense strand, while the S1 antisense strand is also not complementary to the target gene by one nucleotide. HCV6+1 inhibits expression less effectively than HCV5+6, but more effectively than HCV7+8 (Figure 2). In other words, specificity and effectiveness of the expression-inhibiting action of dsRNA depends more on the sequence of the S1 antisense strand than on that of the S2 sense strand.

HCV3+4 (Figure 1) and K3S+K3AS (Figure 2), which serve as the negative controls, led to no and little inhibition of luciferase activity, respectively. The minute inhibition is

nonspecific, as it is not dependent on the dsRNA concentrations used.

The data show that at least two nucleotides in the antisense strand of a dsRNA that are not complementary to an original gene are necessary to prevent inhibition of expression of the original gene. Furthermore, the data show that it is possible to modulate the effectiveness of inhibition of the expression by a dsRNA by lessening the extent of complementarity of the single strands that form the dsRNA.

Patent Claims

1. Use of a double-stranded ribonucleic acid (dsRNA) to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene, whereby an S1 strand of the dsRNA exhibits a region that is complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene.

2. Use of a double-stranded ribonucleic acid (dsRNA) to produce a medicament to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene, whereby an S1 strand of the dsRNA exhibits a region that is complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene.

3. Use in accordance with Claim 1 or 2, whereby the nucleotide that is not complementary to the target gene is not located at the 3'- or 5'-end of the region.

4. Use in accordance with one of the previous claims, whereby the target gene exhibits one or two point mutations not seen in the original gene.

5. Use in accordance with one of the previous claims, whereby the original gene is a proto-oncogene and the target gene is an oncogene derived from it.

6. Use in accordance with one of the previous claims, whereby the cell is a tumor cell.

7. Use in accordance with one of the previous claims, whereby one nucleotide of the region is not complementary to the target gene, and two nucleotides of the region are not complementary to the original gene.

8. Use in accordance with one of the previous claims, whereby at least one base pair within the dsRNA is not complementary.

9. Use in accordance with one of the previous claims, whereby the dsRNA exhibits a single-stranded overhang at least at one end of the dsRNA, consisting of 1 to 4, in particular of 2 or 3 nucleotides.

10. Use in accordance with Claim 9, whereby the dsRNA exhibits the overhang exclusively at one end, in particular at its end exhibiting the 3'-end of the S1 strand.

11. Use in accordance with one of the previous claims, whereby the S1 strand or an S2 strand possibly contained in the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

12. Use in accordance with one of the previous claims, whereby the dsRNA is present in a preparation suitable for inhalation, oral ingestion, infusion, or injection, in particular for intravenous, intraperitoneal or intratumoral infusion or injection.

13. Use in accordance with Claim 12, whereby the preparation consists of the dsRNA and a physiologically tolerated buffer, particularly a phosphate buffered saline solution.

14. Use in accordance with one of the previous claims, whereby dsRNA is present in a physiologically tolerated buffer, particularly a phosphate buffered saline solution, or surrounded by a micellar structure, preferably a liposome, a virus capsid, or a capsoid.

15. Use in accordance with one of the previous claims, whereby dsRNA is administered orally, by inhalation, infusion, or injection, in particular by intravenous, intraperitoneal, or intratumoral infusion or injection.

16. Use in accordance with one of the previous claims, whereby dsRNA is administered to a mammal, preferably a human being, at a maximum dosage of 5 mg, particularly 2.5 mg, preferably 200 µg, most preferably 100 µg per kilogram body weight per day.

17. Medicament to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene, whereby the medicament contains a double-stranded ribonucleotic acid (dsRNA), whereby an S1 strand of the dsRNA exhibits a region that is complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene.

18. Medicament in accordance with Claim 17, whereby the nucleotide that is not complementary to the target gene is not located at the 3'- or 5'-end of the region.

19. Medicament in accordance with Claim 17 or 18, whereby the target gene exhibits one or two point mutations not seen in the original gene.

20. Medicament in accordance with one of the Claims 17 to 19, whereby the original gene is a proto-oncogene and the target gene is an oncogene derived from it.

21. Medicament in accordance with one of the Claims 17 to 20, whereby the cell is a tumor cell.

22. Medicament in accordance with one of the Claims 17 to 21, whereby one nucleotide of the region is not complementary to the target gene, and two nucleotides of the region are not complementary to the original gene.

23. Medicament in accordance with one of the Claims 17 to 22, whereby at least one base pair within the dsRNA is not complementary.

24. Medicament in accordance with one of the Claims 17 to 23, whereby the dsRNA exhibits a single-stranded overhang at least at one end of the dsRNA, consisting of 1 to 4, in particular of 2 or 3 nucleotides.

25. Medicament in accordance with Claim 24, whereby the dsRNA exhibits the overhang exclusively at one end, in particular at its end exhibiting the 3'-end of the S1 strand.

26. Medicament in accordance with one of the Claims 17 to 25, whereby the S1 strand or an S2 strand possibly contained in the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

27. Medicament in accordance with one of the Claims 17 to 26, whereby the medicament exhibits a preparation suitable for inhalation, oral ingestion, infusion or injection, in particular for intravenous, intraperitoneal or intratumoral infusion or injection.

28. Medicament in accordance with Claim 27, whereby the preparation consists of the dsRNA and a physiologically tolerated buffer, in particular a phosphate buffered saline solution.

29. Medicament in accordance with one of the Claims 17 to 28, whereby dsRNA is present in the medicament in a solution, particularly a physiologically tolerated buffer, or surrounded by a micellar structure, preferably a liposome, a virus capsid, or a capsoid.

30. Medicament in accordance with one of the Claims 17 to 29, whereby in each intended dosage unit the dsRNA is present in a quantity that corresponds to a maximum dosage of 5 mg, particularly 2.5 mg, preferably 200 µg, most preferably 100 µg per kilogram body weight.

31. Method to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene, whereby a double-stranded ribonucleic acid (dsRNA) is introduced into the cell, and an S1 strand of the dsRNA exhibits a region that is complementary to the target

gene, in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene.

32. Method in accordance with Claim 31, whereby the nucleotide that is not complementary to the target gene is not located at the 3'- or 5'-end of the region.

33. Method in accordance with Claim 31 or 32, whereby the target gene exhibits one or two point mutations not seen in the original gene.

34. Method in accordance with one of the Claims 31 to 33, whereby the original gene is a proto-oncogene and the target gene is an oncogene derived from it.

35. Method in accordance with one of the Claims 31 to 34, whereby the cell is a tumor cell.

36. Method in accordance with one of the Claims 31 to 35, whereby one nucleotide of the region is not complementary to the target gene, and two nucleotides of the region are not complementary to the original gene.

37. Method in accordance with one of the Claims 31 to 36, whereby at least one base pair within the dsRNA is not complementary.

38. Method in accordance with one of the Claims 31 to 37, whereby the dsRNA exhibits a single-stranded overhang at least at one end of the dsRNA, consisting of 1 to 4, in particular of 2 or 3 nucleotides.

39. Method in accordance with Claim 38, whereby the dsRNA exhibits the overhang exclusively at one end, in particular at its end exhibiting the 3'-end of the S1 strand.

40. Method in accordance with one of the Claims 31 to 39, whereby the S1 strand or an S2 strand possibly contained in the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

41. Method in accordance with one of the Claims 31 to 40, whereby dsRNA is present in a solution, particularly a phosphate buffered saline solution, or surrounded by a micellar structure, preferably a liposome, a virus capsid, or a capsoid.

Summary

The invention concerns the use of a double-stranded ribonucleic acid (dsRNA) to selectively inhibit expression of a given target gene in a cell with a point mutation not found in an original gene, whereby an S1 strand of the dsRNA exhibits a region that is complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and at least one nucleotide more than to the target gene is not complementary to the original gene.

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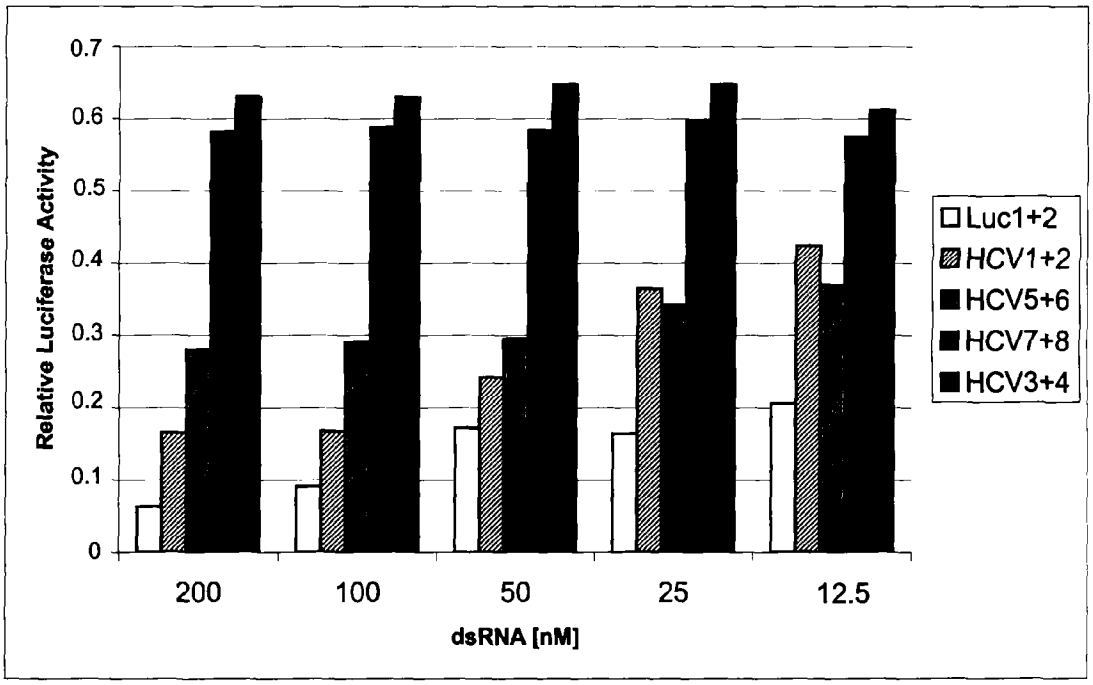
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative Luciferase Activity
200
100
50
25
12.5
dsRNA [nM]
Luc1+2
HCV1+2
HCV5+6
HCV7+8
HCV3+4

Fig. 1

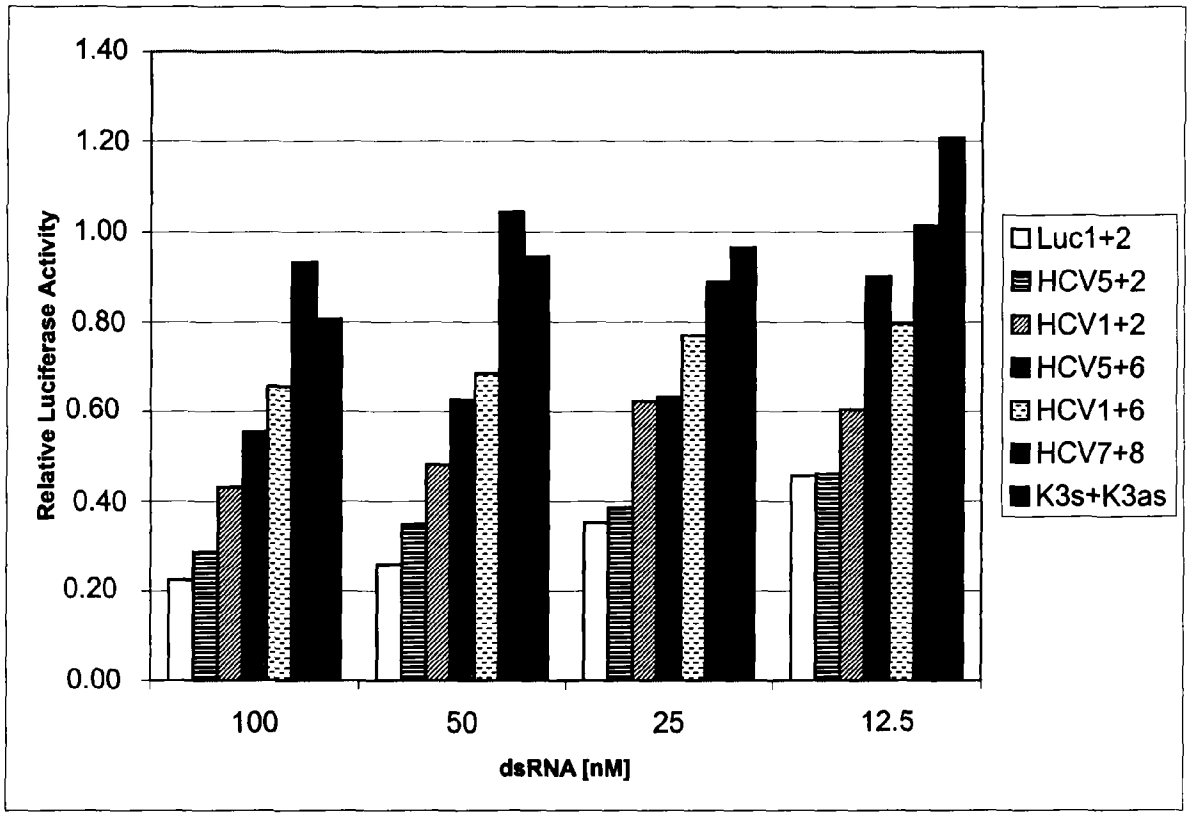
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Relative Luciferase Activity
100
50
25
12.5
dsRNA [nM]
Luc1+2
HCV5+2
HCV1+2
HCV5+6
HCV1+6
HCV7+8
K3s+K3as

Fig. 2